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Liver Transplantation After *Ex Vivo* Normothermic Machine Preservation: A Phase 1 (First-in-Man) Clinical Trial

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The number of donor organs suitable for liver transplantation is restricted by cold preservation and ischemia–reperfusion injury. We present the first patients transplanted using a normothermic machine perfusion (NMP) device that transports and stores an organ in a fully functioning state at 37°C. In this Phase 1 trial, organs were retrieved using standard techniques, attached to the perfusion device at the donor hospital, and transported to the implanting center in a functioning state. NMP livers were matched 1:2 to cold-stored livers. Twenty patients underwent liver transplantation after NMP. Median NMP time was 9.3 (3.5–18.5) h versus median cold ischaemia time of 8.9 (4.2–11.4) h. Thirty-day graft survival was similar (100% NMP vs. 97.5% control, p = 1.00). Median peak aspartate aminotransferase in the first 7 days was significantly lower in the NMP group (417 IU [84–4681]) versus (902 IU [218–8786], p = 0.03). This first report of liver transplantation using NMP-preserved livers demonstrates the safety and feasibility of using this technology from retrieval to transplantation, including transportation. NMP may be valuable in increasing the number of donor livers and improving the function of transplantable organs.

Abbreviations: AST, aspartate transaminase; DBD, donation after brain death; DCD, donation after circulatory death; IRI, ischemia–reperfusion injury; IVC, inferior vena cava; MELD, model for end-stage liver disease; NHSBT, National Health Service Blood and Transplant; NMP, normothermic machine perfusion

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Introduction

The availability of donor organs for transplantation is restricted by the limitations of current cold preservation techniques. Despite rising numbers of organ donors in many countries, the gap between demand and availability of donor livers is increasing, with waiting lists and pre-transplant mortality growing in many countries (1,2). On April 1, 2014, 552 patients were on the UK liver transplant waiting list. During the previous 12 months, 20% of patients died while waiting or were removed from the list (typically having become too sick to transplant) (3). Despite this, only 65% of solid organ donors culminated in a liver transplant (3).

Much of the increase in deceased donor numbers is in donors that would once have been declined as unsuitable—including older donors and those with medical comorbidities (cardiovascular disease, diabetes, obesity) (3). Also, organ donation is increasingly offered following cardiovascular/circulatory, rather than neurological, determination of death: this inevitably implies a period of warm ischemic injury prior to preservation (3). The use of these marginal organs is associated with a much higher risk of immediate graft failure and later complications (4,5). Notably, while 87% of all donation after brain death (DBD) livers in the United Kingdom were utilized, only 28% of donation after circulatory death (DCD) livers were transplanted (3).

The standard technique for the preservation of donor organs between recovery and implantation is static cold storage in a specialist preservation solution. Although effective for ideal donor organs, this method is less suitable for marginal (high-risk) organs, not only because such organs experience greater ischemia–reperfusion effects, but also because the lack of an effective means of viability assessment is so much more problematic in this group. Investigators around the world are exploring novel preservation methods in an attempt to enable greater use of such high-risk organs without compromising outcomes. Recent advances include the demonstration of

the benefit of cold continuous machine perfusion for the kidney (6) and the first use of a similar strategy in the liver (7,8). Early clinical investigations by the Zurich group suggest that oxygenated hypothermic liver perfusion at the end of cold preservation is beneficial (9,10). However, there has been no widely adopted change in clinical practice since the introduction of University of Wisconsin solution in the late 1980s.

There is mounting evidence that only perfusion under more physiological conditions of temperature and oxygen delivery will enable a step change in the utilization of marginal donor organs. Recent clinical studies have tested varying periods of normothermic perfusion ("reconditioning") of donor organs transported in a cold state for the kidney (11) and lung (12,13). However, these studies have not achieved normothermic preservation throughout the period from explantation to implantation. The feasibility of maintaining physiological temperature throughout the period of lung preservation was recently demonstrated (14), but clinical success has not yet been reported in the context of other organs. We have previously demonstrated in a pig model that even a short (4 h) period of cold preservation is markedly deleterious to the liver following an ischemic injury and concluded that, if normothermic machine perfusion (NMP) is to be beneficial in the transplantation of marginal donor organs, then the technology must be transportable to the donor hospital (15).

We present here, in a Phase 1 trial designed to test safety and feasibility rather than efficacy, the results of the first clinical series of transplants carried out using a novel normothermic liver perfusion device that enables transport, storage, and assessment of a liver in a fully functioning state.

Methods

In this Phase 1, nonrandomized, prospective trial, the outcomes of the recipients of 20 consecutive NMP donor livers were compared to those of matched control patients who received conventionally cold-stored donor livers. Approvals were obtained from National Health Service Blood and Transplant (NHSBT), National Research Ethics Committee, and the Medicines and Healthcare Products Regulatory Authority. The trial was registered with the ISRCTN (14355416).

Patient and donor selection

Adult patients with end-stage liver disease on the King's College Hospital and University Hospital Birmingham liver transplant waiting list were approached for consent to take part in the study. The families of suitable organ donors were approached for consent. All adult donor organs, aged over 18 years, including DBD and DCD were potentially eligible, except those undergoing splitting for two recipients. All adult recipients, aged over 18 years, were potentially eligible, except those undergoing transplantation for fulminant liver failure (because of the marginal of non-graft-related mortality) or transplantation of more than one organ.

Matching

Patients undergoing NMP liver transplantation were matched retrospectively 1:2 to patients undergoing transplantation of conventional cold-stored livers at the same centers between January 2011 and December 2013 (a broader time span than the trial recruitment in order to achieve the desired level of matching). Anonymized, matched control patients were identified by applying the following criteria hierarchically, in the following order: (i) graft type (DBD, DCD); (ii) donor age (within 5 years); (iii) recipient MELD (model of end-stage liver disease) score (within 2 points); (iv) recipient age (within 10 years). Matching criteria limits were extended when no suitable matches were identified. Ten donor livers were within standard criteria and the other 10 donor livers were specifically selected as high-risk, using criteria based on the Eurotransplant Donor Risk Index (16).

End-points

The primary end-point was 30-day graft survival. Secondary end-points included biochemical measures of liver function/injury (bilirubin, aspartate aminotransferase [AST], alkaline phosphatase [ALP], international normalized ratio [INR]) during the first 7 days, patient and graft survival, and graft function at 6 months. Early allograft dysfunction was defined by the occurrence of one or more of the following: bilirubin >170 μmol/L on day 7 posttransplant; INR >1.6 on day 7 posttransplant; peak AST >2000 IU/L within the first 7 days posttransplant (17).

Analysis

Statistical analysis was conducted using SPSS® 22 (IBM®, New York), with data expressed as medians and ranges. Continuous numerical data were compared using a Mann–Whitney U test for nonparametric data or Kruskal–Wallis test of multiple variance; Fisher's exact test was used to compare categorical data. For categorical outcomes, absolute differences between the NMP and control groups, expressed as percentage points with 95% confidence intervals (CI), are provided. Differences were considered to be of statistical significance when a p-value of <0.05 was achieved.

Machine perfusion

When a suitable donor liver with research consent was allocated to a consenting recipient, the perfusion device was transported to the donor hospital. The perfusion team prepared the device and set up the surgical back-table during the retrieval process. Standard multiorgan retrieval was carried out with the addition of *in-situ* cholecystectomy (to reduce the risk of bleeding during perfusion). The liver was cooled *in situ* with University of Wisconsin solution and transferred to the back-table.

The suprahepatic inferior vena cava (IVC) was prepared by excising attached diaphragmatic tissue and oversewing the orifices of the phrenic veins, and then closed using a linear vascular stapler (Covidien, Hampshire, UK). The infrahepatic IVC was cannulated (28F Sorin, Gloucester, UK). The hepatic hilum was dissected, taking care to ligate all tributaries. Cannulae were secured in the portal vein (24F Sorin), celiac artery (10F Sorin), and common bile duct (12–18Fr Summit Medical, Cheltenham, UK). In three cases, an accessory right hepatic artery was anastomosed to the gastroduodenal artery. The liver was flushed with 500 mL colloid solution (Gelofusine®, B Braun, South Yorkshire, UK) to remove preservation solution, and then transferred to the perfusion device.

The OrganOx *metra* liver perfusion device (Figure 1) provides automated pumping, oxygen/air delivery, and heat exchange, in order to maintain the perfusate at normal temperature, within physiological ranges for pO_2, pCO_2, pH, and at physiological pressures in the vascular inflows and outflow of the liver (hepatic artery pressure 60 to 75 mmHg; IVC pressure (−1 to 2 mmHg.). The portal pressure was not monitored (it is effectively fixed by the height of the portal venous reservoir), but portal flow is continuously measured. Hemodynamic parameters and blood gas data are

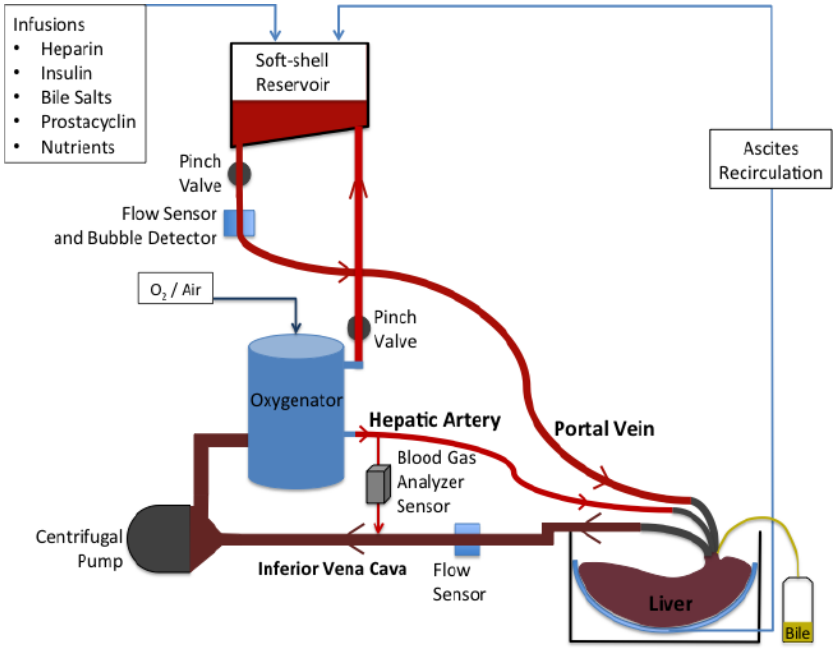

Figure 1: Schematic representation of the OrganOx *metra* circuit.

continuously recorded during preservation. Cannulation of the bile duct enables collection and automated monitoring of hourly bile production. The machine also continuously infuses (i) bile salt (sodium taurocholate, New Zealand Pharmaceuticals, Palmerston North, New Zealand); (ii) insulin (Actrapid®, Novo Nordisk, West Sussex, UK); (iii) heparin (CP Pharmaceuticals, Wrexham, UK); (iv) prostacyclin (Flolan®, Glaxo, Middlesex, UK). A variable rate infusion of glucose and amino acids (Nutriflex, B Braun, Sheffield, UK) is regulated by 4-hourly manually inputted glucose levels.

The device was primed with three units of packed red blood cells, sourced from the blood bank and cross-matched to the donor, and one unit of colloid solution (Gelofusine®, B Braun), with addition of calcium gluconate (B Braun), heparin (CP Pharmaceuticals), cefuroxime (GSK), and 30 mL of sodium bicarbonate (B Braun). During priming, the perfusate was allowed to reach operating conditions: temperature (37°C); pO_2 (12 kPa); pCO_2 (5 kPa); pH (7.35). The cannulated organ was then connected and blood flow started. Once perfusion was established, minor bleeding points were controlled surgically and the liver container was then closed.

Transport and implantation

The organ was transported by road to the transplant hospital and remained on the perfusion device until the transplanting team was ready to implant the organ. Perfusion was then stopped and the organ was cooled by rapid perfusion of 2 L of cold HTK solution (Custodiol®-HTK, Essential Pharmaceuticals, Ewing, NJ). The cannulae were removed and the organ was transferred to the recipient for immediate revascularization, using the unit's standard surgical technique. Postoperative management was conducted according to standard local protocols, which included tacrolimus-based immunosuppression.

Results

Donor and recipient characteristics

Between February and December 2013, 20 patients underwent liver transplantation using donor organs preserved from recovery to implantation by normothermic perfusion (Table 1). There were no device failures leading to organs not being transplanted. The cases reported here represent 20 consecutive perfusions, without omission. Sixteen livers (80%) were from DBD and four (20%) were from DCD (Maastricht category III: circulatory arrest following withdrawal of support) donors. The indication for transplantation was chronic liver failure except in one recipient who underwent retransplantation for hepatic artery thrombosis. The underlying etiology of liver disease was the following: hepatitis C virus infection (n = 6); alcoholic liver disease (n = 5); primary sclerosing cholangitis (n = 3); primary biliary cirrhosis (n = 2); α1-antitrypsin deficiency (n = 1); nonalcoholic steatohepatitis (n = 1); chronic autoimmune hepatitis (n = 1); other cholangiopathic disease (choledocholithiasis and biliary cirrhosis) (n = 1).

Matched control patients were identified as described above; to find two matched controls, preset criteria were extended in some cases, as follows: donor age in liver 17 (85 years, matched liver 79 years), and 19 (27 years, matched 33 years); recipient age in liver 8; MELD score in livers 14 and 19.

Median donor age was 58.0 (21–85) years in NMP versus 58.5 (21–82) years in matched controls (p = 0.93). Median recipient age was 54.4 (33–66) years in NMP versus 55.0 (27–65) years in matched controls (p = 0.99). In DCD transplants, donor median warm ischemic time was 21 (range 14–31) min in NMP versus 15 (9–23) min in the matched controls (p = 0.53). Median recipient

Table 1: Characteristics of normothermic liver perfusion (NMP) and control livers

	Graft type		Donor age (years)		MELD		Recipient age (years)		Preservation time (h)	
Patient	NMP	Control	NMP	Control	NMP	Control	NMP	Control	NMP	Control
1	DBD	DBD	62	61	18	18	62	61	4.5	7.83
		DBD		59		18		59		9
2	DBD	DBD	44	42	25	25	54	48	9.87	5
		DBD		46		23		46		9.5
3	DBD	DBD	64	68	Re-Tx HAT	Re-Tx CR	43	48	10.75	13.35
		DBD		65		ReTx RD		40		8.78
4	DBD	DBD	41	46	27	23	38	46	3.5	9.5
		DBD		42		25		48		5
5	DBD	DBD	53	56	18	20	54	53	6.15	10.1
		DBD		55		16		58		9.38
6	DBD	DBD	50	49	12	13	61	62	14.1	9.83
		DBD		49		13		62		10.5
7	DBD	DBD	68	66	9	8	46	47	15.8	9
		DBD		66		10		45		8.16
8[1]	DBD	DBD	77	73	7	6	47	59[1]	9.75	4.23
		DBD		72		8		35[1]		8.33
9	DBD	DBD	59	59	11	7	60	61	12.5	5.83
		DBD		56		14		58		8
10	DBD	DBD	78	79	15	15	62	60	11.58	5.5
		DBD		75		17		57		6.67
11	DCD, 14 min	DCD, 22 min	64	65	11	9	52	51	5.92	5.6
		DCD, 23 min		65		12		48		9.23
12	DBD	DBD	46	46	14	13	58	60	8.87	6.4
		DBD		45		16		65		7
13	DBD	DBD	61	64	18	19	55	50	4.75	7.6
		DBD		63		19		52		7.45
14[1]	DBD, mod steatosis 3 kg	DBD	47	48	11	14	57	62	8.83	7.2
		DBD		48		16[1]		56		10.2
15	DBD, fibrotic, retrieval ALT 1300	DBD	21	21	16	18	48	49	7.17	11.42
		DBD		23		18		44		10.08
16	DCD, 27 min	DCD, 20 min	53	58	11	12	66	62	5.5	5.78
		DCD, 23 min		56		7		63		8.87
17[1]	DBD	DBD	85	82	12	8	54	54	18.5	6.17
		DBD		79[1]		14		60		5.5
18	DCD, 31 min	DCD, 18 min	67	65	12	13	64	62	17.82	9.83
		DCD, 23 min		64		11		47		7.9
19[1]	DBD, fibrotic, retrieval ALT 2300	DBD	27	33[1]	12	17[1]	33	27	10.5	7.62
		DBD		26		16		37		11.08
20	DCD, 15 min	DCD, 10 min	57	57	9	10	57	54	8	5.78
		DCD, 9 min		59		7		61		5.97

For graft type, numbers expressed adjacent to DCD indicate warm ischemia time in minutes.
MELD, model for end-stage liver disease; DBD, donation after brain death, DCD, donation after circulatory death; Re-Tx, retransplantation; HAT, hepatic artery thrombosis; CR, chronic rejection; RD, recurrent disease; ALT, alanine aminitransferase.
[1]Livers where the matching criteria had to be extended.

MELD was 12 (7–27) in NMP versus 14 (6–25) in matched controls ($p = 0.55$).

Assessment during NMP

Median NMP time was 9.3 (range from 3.5 to 18.5) h (Figure 2A). Median cold ischemia time in the matched controls was 8.9 (range 4.2–11.4) h (Table 2). The period of NMP was governed by logistic considerations (mainly other transplants).

There was evidence of stable hemodynamic, synthetic, and metabolic function throughout all perfusions (Figure 2) with maintenance of pH between 7.2 and 7.4 (Figure 2C), without pharmacological correction. Bile production commenced after the first hour and was maintained throughout NMP (Figure 2B). Hepatic arterial and portal venous flows were consistent throughout (Figure 2D).

Outcomes (Table 2)

All grafts and patients in the NMP group survived the first 30 days but one recipient of a DBD liver in the matched control group died on day 0 from a cardiovascular

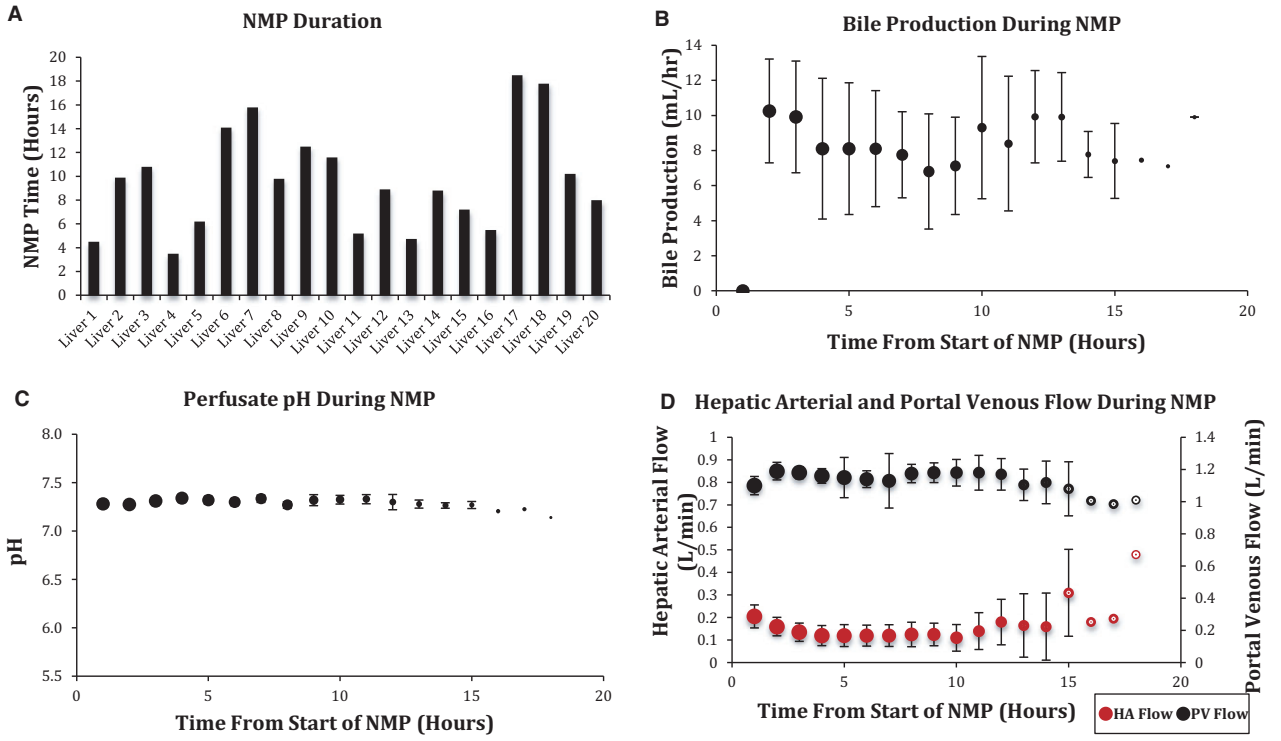

Figure 2: Assessment during normothermic machine perfusion (NMP). (A) NMP duration; (B) bile production; (C) perfusate pH during NMP; (D) hepatic arterial and portal venous flow during NMP.

Table 2: Clinical outcomes of normothermic machine perfusion (NMP) and control livers

	Total			
Outcomes	NMP (n = 20)	Control (n = 40)	Risk ratio/effect size (95% CI)	p-value
30-day graft survival, n (%)	20 (100)	39 (97.5)	1.03 (0.98–1.08) RR	1.00
PNF, n (%)	0	0		1.000
EAD, n (%)	3 (15)	9 (22.5)	0.67 (0.20–2.19) RR	0.734
Peak AST within 7 days (IU/L), median (range)	417 (84–4681)	902[1] (218–8786)	−0.44 (−0.98 to 0.11) ES	0.034
Bilirubin on day 7 (μmol/L), median (range)	25 (8–211)	30[1] (9–221)	−0.23 (−0.77 to 0.32) ES	0.203
INR on day 7, median (range)	1.05 (0.88–1.40)	1.03 (0.90–2.22)[1]	−0.16 (−0.70 to 0.38) ES	0.922
ALP on day 7 (U/L)	245 (81–568)	243 (76–743)[1]	−0.11 (−0.65 to 0.43) ES	0.798
ITU stay (days), median (range)	3 (1–8)	3 (1–41)[1]	−0.42 (−0.96 to 0.13) ES	0.459
Hospital stay (days), median (range)	12 (6–34)	14 (8–88)[1]	−0.44 (−0.98 to 0.11) ES	0.100
30-day mortality (%)	0 (0)	1 (2.5)		1.000
6-month survival, n (%)	20 (100)	39 (97.5)	1.03 (0.98–1.08) RR	1.000

ALP, alkaline phosphatase; AST, aspartate aminotransferase; INR, international normalized ratio; ITU, intensive therapy unit; DBD, donation after brain death; DCD, donation after circulatory death; PNF, primary nonfunction; EAD, early graft dysfunction; RR, relative risk; ES, effect size; CI, confidence interval.
[1]N = 39 as 1 death on day 0.

event (100% NMP vs. 97.5% Control, Absolute Difference −2.5, 95% CI −7.5–2.5; p = 1.00). There was a statistically significant difference in peak AST levels (417 vs. 902 IU/L, p = 0.034), numerically more pronounced in the DCD cohort (422 vs. 1894 IU/L, p = 0.283). There was no primary nonfunction in either group. Three patients (15%) demonstrated early graft dysfunction (EAD) in the NMP group compared to nine (23%) in the control group. This difference was more pronounced in the DCD subset (one [25%] vs. four [50%] patients). EAD in the NMP group was due to the following: day 7 bilirubin of 211 (liver 8, donor age 77); peak AST of 2158 IU/L (liver 15 prerecovery AST of 1300 IU/L); peak AST of 4681 IU/L (liver 16, DCD, age 53, warm ischemia time 27 min). Median intensive therapy unit and hospital stays were similar between the two groups overall and when analyzed as DBD and

Table 3: Complications of normothermic machine perfusion (NMP) livers

Patient	Graft type	NMP complications
1	DBD	
2	DBD	Anastomotic biliary stricture, stented
3	DBD	Anastomotic biliary stricture, biliary sepsis, stented
4	DBD	
5	DBD	
6	DBD	
7	DBD	*Escherichia coli* sepsis and acute kidney injury
8	DBD	
9	DBD	
10	DBD	Anastomotic biliary stricture, stented
11	DCD	Urinary sepsis and diabetes
12	DBD	
13	DBD	
14	DBD	
15	DBD	
16	DCD	
17	DBD	Death from recividism at 9 months
18	DCD	
19	DBD	Sepsis
20	DCD	CMV +ve donor into CMV −ve recipient; recipient converted

DBD, donation after brain death, DCD, donation after circulatory death; CMV, cytomegalovirus.

DCD subsets. All patients and grafts in the NMP group survived 6 months. One-year patient survival in the NMP group was 95% (one death at 9 months as a result of alcohol recividism).

Figure 3 demonstrates actual biochemical parameters for each NMP liver compared to matched controls. Differences are seen in peak AST and bilirubin, particularly in the last 10 liver grafts (a higher-risk group).

Four anastomotic biliary strictures in the trial patients underwent stenting, all in recipients of DBD grafts (Table 3), with the following primary diagnoses: one cholangiopathic disease, one retransplant for hepatic artery thrombosis, one alcoholic liver disease, and one primary biliary cirrhosis. There were no vascular complications within this study. One trial patient developed postoperative hepatic parenchymal infarcts, which resolved on subsequent computed tomography imaging. Notably, this patient received a liver with poor NMP arterial flow. One patient developed noncirrhotic portal hypertension with ascites but well maintained synthetic function. A 6-month posttransplant biopsy demonstrated perivenular fibrosis, mild portal-lobular hepatitis, and injury to small interlobular bile ducts attributed to a veno-occlusive disease in the absence of alternative overt pathology. Perfusate cultures (not carried out routinely) were all negative for bacterial growth. No significant difference was seen in the pre- and postperfusion biopsies, with all showing scattered neutrophils in keeping with mild preservation injury.

Safety, feasibility, and logistics

All livers selected for this trial underwent NMP throughout the period of preservation. The results reported were of 20 consecutive NMP livers with no exclusions (e.g. due to aborted perfusions). No livers were excluded due to perfusions. The 40–60 min of back-table preparation at the donor hospital occurred in parallel with the 30 min required to prime the normothermic perfusion device. Connection of the organ to the device and confirmation of stable flows typically took less than 15 min. With the exception of one liver (retrieved within the transplanting center), all grafts were then transported by road, with journey times of up to 3 h. A VW Transporter minibus with mains power outlet was used for transport. One member of the team oversaw the perfusion parameters and the device function throughout the perfusion. The only technical complication during transport was an air-lock in the fluid sensing system, which necessitated a brief stop during transit to rectify the problem. Subsequent minor modification of the circuit design prevented any recurrence; there were no other transport-related complications and no liver became unsuitable for transplantation due to perfusion problems. The importance of meticulous back-table preparation of the liver was noted at an early stage, in order to avoid the need for hemostatic procedures once on the device. Although the need did not arise, cold preservation solution was always carried, with a tubing set for quick connection to the cannulated liver to enable rapid conversion to cold storage. Although the device is also designed to be suitable for transport in small planes (as used in organ retrieval practice), this was not tested in this study.

Discussion

This is the first report of normothermic perfusion in clinical liver transplantation. This preservation methodology potentially reduces the risk inherent in transplanting marginal donor organs—a key challenge in an era when donor numbers are increasing but many organs are not transplanted.

The limitations of cold storage of marginal livers are well recognized and are the primary reason for the poor utilization of such organs, illustrated by the 28% transplant rate of DCD livers in the United Kingdom (NHSBT data) (3). The methodology used in this study does not totally eliminate the exposure of the graft to cold ischemia, but limits this to brief periods immediately before and after NMP. Large animal experimental data demonstrate that NMP allows successful transplantation of livers damaged by hypoxia (a model of DCD) that do not survive with conventional cold storage (18). Also, preliminary data suggest that NMP may be effective in allowing

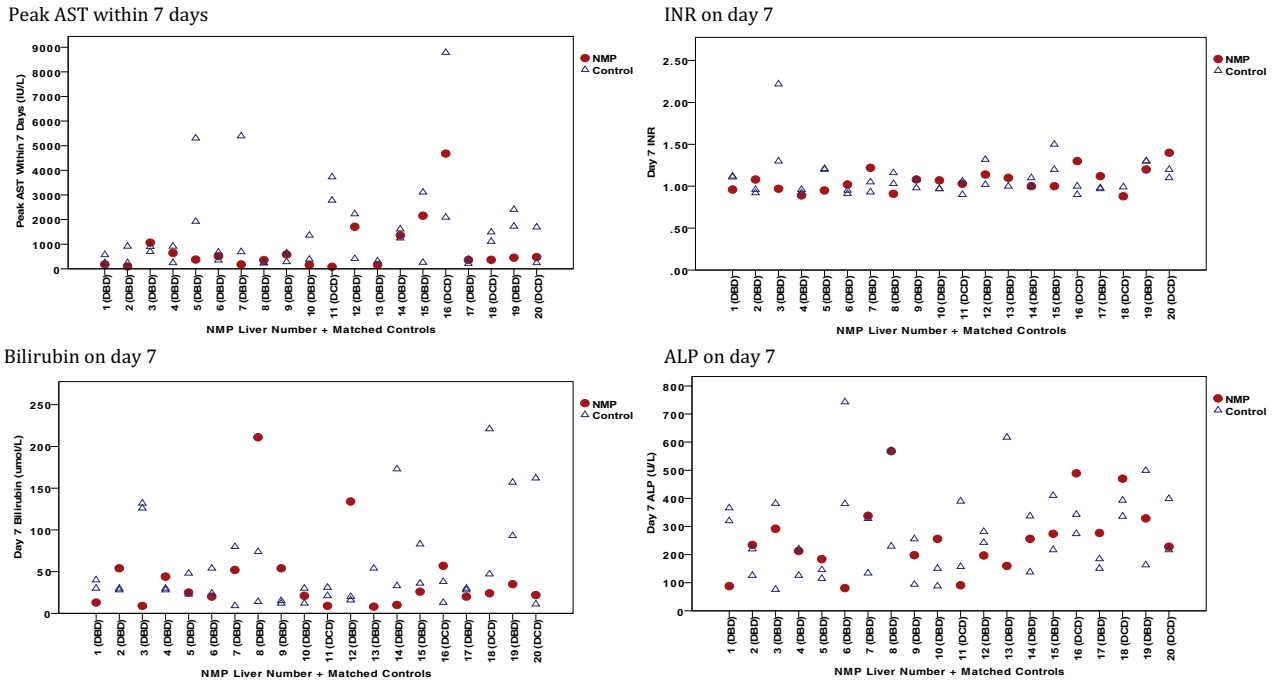

Figure 3: Postoperative biochemical results of the normothermic machine perfusion (NMP) and control livers. AST, aspartate transaminase; INR, international normalized ratio; ALP, alkaline phosphatase.

resolution of the intracellular fat deposits in steatotic livers that are also associated with poor outcomes (19)—although no evidence exists as to whether this improves survival. What will only be shown in clinical (as opposed to experimental) trials is whether this new preservation technology is superior in the broader range of clinical scenarios that comprise the marginal donor.

This Phase 1 study was designed for assessment of both safety and logistic factors. The results showed the procedure is feasible and safe. All livers enrolled into the study were successfully transplanted with 100% 30-day recipient and graft survival. This trial was not designed to demonstrate efficacy: The inclusion of matched control patients allows preliminary comparisons to be made and will be helpful in the design of future comparative studies. In addition to safety, this study provided the opportunity to test vital logistic issues—transportability and usability.

Although the results in the 20 patients reported here showed no statistical difference in the primary outcome, with an important caveat regarding the small numbers, it suggests that NMP halves peak transaminase, a surrogate marker for graft function (20,21) and preservation injury (22,23). As noted above, the control group covered a longer time period than the intervention group to allow adequately matched control patients to be identified in the two liver transplant units concerned. The entry criteria for the study were broad and reflected clinical reality: although 10 donors were deliberately selected as being well within current liver transplant criteria, as the trial progressed, several donor organs were selected that were close to the extreme of (although still within) what would be accepted for transplantation in current practice. These included three donors aged between 77 and 85 years; a 3.1 kg steatotic liver from a 140 kg donor; and two donors with a peak prerecovery transaminase of >1000 IU/L. Preservation durations of up to 24 h (substantially longer than standard clinical practice) were approved by the Ethics Committee, supported by the extensive published preclinical work underpinning the preservation technology (18). Following five initial preservations not exceeding 12 h, longer periods of preservation were also carried out, up to a maximum of 18.5 h. This progression of indications aligns with the recommendations of the IDEAL collaboration (24).

A major potential advantage of NMP is the measurement of organ function during storage. Experimental evidence suggests that the viability of the donor organ can be predicted by hemodynamic, metabolic, and synthetic (e.g. bile output) parameters during perfusion and that this may allow clinically useful viability testing (25). This hypothesis is supported by a recently published study of perfused discarded livers, suggesting that bile output and other metabolic parameters may differentiate viable from nonviable livers (26). If this is validated in a clinical environment, then the risk associated with marginal organs will be reduced, by enabling clinicians to accept an organ

provisionally, secure in the knowledge that further information will become available before committing the patient to the risk of a transplant. The data reported here do not address this issue, which will require much larger studies.

Similarly, the reported data, limited by size, do not address the issue of ischemic cholangiopathy, a complication strongly associated with DCD liver transplantation (27,28). Experimental data from large animal studies, specifically investigating biliary epithelial recovery following a period of warm ischemic and NMP, suggest that warm oxygen delivery may provide a critical advantage (29,30). This is a key issue in the quest to increase the utilization of DCD organs in liver transplantation and will only be addressed in the context of a randomized trial with longer-term follow-up of patients. The anastomotic biliary strictures seen in our series occurred early and we believe are more likely to be complications of surgery than preservation (31); indeed, two of these occurred in patients who might have been managed with hepaticojejunostomy.

Having now demonstrated safety and usability in the clinical context, the next requirement is to test whether this technology is superior to existing standard of care (static cold storage). A multicenter randomized controlled trial is planned, powered to demonstrate superiority in surrogate markers of survival (20,21). It is vital that a trial of this sort is carried out at this early stage—while equipoise exists. To test the potential of NMP to increase the donor pool, there is also a case for a noninferiority trial in which marginal organs, treated with the new method, are compared with standard criteria donor organs treated with conventional preservation.

The results of this first use of NMP of the liver provide data on proof of concept, safety, and logistics, which will support trials to test efficacy and health-economic benefits. We believe that normothermic preservation may substantially improve organ utilization.

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The funder for this study, OrganOx Ltd, is also the Sponsor. The decision to publish the outcomes of the study in full was agreed by all parties at the time of engagement of the two independent clinical trial centers, King's College Hospital and University Hospital Birmingham. To avoid bias, the Sponsor subcontracted all auditing and monitoring responsibilities to the Clinical Trials and Research Governance Unit, University of Oxford. OrganOx was directly responsible for the collection, analysis, and interpretation of organ perfusion data during preservation. All donor, recipient, and clinical outcome data were entered onto an independent database by clinical research nurses employed by King's College Hospital and University Hospital Birmingham. The database was constructed by Medrio, a San Francisco based company providing software for clinical research. R.R. was the only author to have direct read-only access to the study database and carried out the analysis as part of her doctoral degree work at the University of Oxford. Every liver that was entered into the trial has been reported without any exclusion of data. All authors independently contributed to the interpretation of the data and writing of the manuscript, and approved the final version prior to submission. The authors wish to acknowledge the support of Dr Les Russell, Dr Colin Story, and Dr Toni Day at OrganOx Limited.

Author Contributions

R.R., C.C., and P.F. had the main responsibility of writing the manuscript, with input from all other authors. R.R. and D.H. analyzed the results. W.J., H.M., N.H., D.M., T.P., and A.Q. were study site investigators.

Disclosure

The authors of this manuscript have conflicts of interest to disclose as described by the *American Journal of Transplantation*. There are two groups of authors in the present study: those who were responsible for carrying out the organ retrievals and transplants and managing the patients, and those who were responsible for the development and operation of the novel perfusion device. The members of the research team who were responsible for the organ perfusions all have associations with the device manufacturer (P.F. and C.C.) in addition to being full-time academics at the University of Oxford, receive consultancy payments as nonexecutive medical and technical directors of OrganOx, and are shareholders. R.R., D.H., and T.V. received consultancy income from OrganOx for assisting with the design and testing of the normothermic liver perfusion device and for carrying out normothermic organ preservation out-of-hours. All clinical data were acquired as part of an MHRA-regulated Phase-1 clinical study, and none of the clinicians who were responsible for organ selection, transplantation, and patient management (W.J., H.M., T.P., D.M., N.H., A.Q.) have any conflicts of interest to disclose.

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